Effect of serine residue on the effectiveness of cationic polypeptide-based gene delivery

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ABSTRACT

Poly-L-lysine (pL) was chemically modified based on two essential features which we recently reported and subjected to the gene-transfer experiment in vitro. Introduction of 25 mol% serine residue to pL slightly enhanced the gene expression level, while trimethylation of ε-ammonium groups of lysine did not. Only when pL was modified in both way, giving N’-trimethyl poly(lysine-co-serine), markedly enhanced gene expression was observed. The cellular uptake and localization of DNA in the cells were similar for each cationic polypeptide. DNA forming complex with the polypeptides containing serine residue was found to be well transcribed in in vitro transcription/translation system, suggesting the hydrophilic nature may allow polypeptide/DNA complexes to be recognized by the transcriptional factors and lead the subsequent effective gene expression.

INTRODUCTION

Cationic polypeptides have been widely studied as biodegradable gene carriers for transfecting foreign genes into mammalian cells in vitro. Among them, poly-L-lysine (pL) is most widely used as base material and conjugated to various biological signals such as proteins, peptides, carbohydrates, which promote the cellular recognitions. However, poor gene-transferring ability of pL itself is well known. It is then important to enhance the gene-transferring ability of pL by its chemical modification.

Plank et al. reported the physicochemical properties of complexes of branched cationic polypeptides with various sequences and DNA. However, the role of such physicochemical properties of polypeptide/DNA complexes during the DNA delivery pathway is unclear.

To date, we evaluated various water-soluble polycations in transfecting DNA to mammalian cells and found that the important features of gene-carriers are their tertiary or quaternary ammonium groups and non-ionic hydrophilic groups such as hydroxyl groups or amide groups. In the present study, chemically modified pL with quaternary ammonium groups and hydroxyl groups were synthesized and used for the gene transfection in vitro, and the key factors influencing transfection efficacy investigated stepwise during the delivery processes.

RESULTS AND DISCUSSION

PL and poly(lysine-co-serine) (pLS), which is a random copolymer of L-lysine and serine with the composition of 3:1, were used for gene-transfer experiment to study the effect of side hydroxyl groups. Trimethyl polylysine (ptmL) and Trimethyl poly(lysine-co-serine) (ptmLS) were prepared by methylation of the ε amino groups in lysine residues of pL and pLS with dimethylsulfate in order to increase their basicity. The complete metylation was confirmed by amino acid analysis.

Plasmid DNA with enhanced green fluorescence protein (EGFP) genes was transferred into COS-1 cells by osmotic shock procedure using these carrier polypeptides. The transient expression was evaluated as the percentage of the number of EGFP-expressing cells emitting under the fluorescent microscope (ex.:488nm and em.:507nm) to the total cell number. The transient expression of EGFP gene introduced with these polypeptides at the different C/A ratio (ratio of cationic groups of cationic polypeptide to anionic groups of DNA) was shown in Table 1. PL and ptmL showed no transient expression of EGFP irrespective of the C/A ratio. On the other hand, pLS and ptmLS having hydroxyl groups showed enhanced transient expression of EGFP. The highest EGFP expressing was obtained in the case of ptmLS having both of quaternary ammonium groups and hydroxyl groups. These results indicate the important role of serine residue in the polypeptide-based gene delivery and strongly support our previous finding.
Table 1. Transient expression of EGFP gene introduced with various cationic polypeptides at various C/A ratio.

<table>
<thead>
<tr>
<th>polypeptide</th>
<th>Transient expression (%)</th>
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<tbody>
<tr>
<td></td>
<td>C/A=3</td>
</tr>
<tr>
<td>pL</td>
<td>0</td>
</tr>
<tr>
<td>ptmL</td>
<td>0</td>
</tr>
<tr>
<td>pLS</td>
<td>0.43</td>
</tr>
<tr>
<td>ptmLS</td>
<td>0.68</td>
</tr>
</tbody>
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a) Cationic groups of cationic polypeptide / anionic groups of DNA

FITC labeled oligo DNAs (F-DNAs) transferred into the cells by the same method was observed under the fluorescent microscope, and the amount of ingested F-DNAs was evaluated by measuring the fluorescence intensity of the cell lysate in 2% SDS.

The amount of F-DNAs taken up by cells was increased with increased C/A ratio and reached plateau irrespective of used polypeptides (Figure 1). In the case of pLS and ptmLS, the maximum expression was seen at C/A=10, which is in good agreement with the transient expression. However, in the case of ptmLS, which induced the highest gene expression, the lowest DNA ingestion was observed. It suggests that the serine residue does not play an important role in the step of cell/DNA interaction but may be required in the following step. The intracellular localization of F-DNAs introduced into the cells using pL and pLS is shown in Figure 2. F-DNA was greatly accumulated into nucleus in the both cases. These data suggests that the cellular uptake and localization of DNA are not the conclusive factor for DNA expression in polypeptide-based gene delivery. Accordingly, the reason for the difference in the transient expressions may be in the recognition step of the complexes by transcriptional factor. Then, transcriptional activity of the polypeptides/DNA complexes encoding luciferase gene was evaluated in in vitro transcription/translation system. DNA forming complex with pLS or ptmLS was found to be well transcribed, and the luciferase activity was 3.48 and 2.09 times larger than pL and ptmL, respectively. Although its mechanism is unclear, the serine residue of pLS and ptmLS must inhibit the compaction of the complexes, and the resulting swollen complexes can easily be transcribed.

REFERENCES